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Prezelin, Barbara B., Triplett, Edward L.

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Our goal is to continue to use biotechnological techniques to study the genetic bases of light- and nutrient-regulation of photosynthetic light-harvesting complexes in marine dinoflagellates. We chose the peridinin-chlorophyll a-protein (PCP) complexes of dinoflagellates as a model system for proposed genetic analyses. Since these phytoplankton are a spectral representative of the large group of marine algae whose light-harvesting components are dominated by blue-light absorbing xanthophylls, knowledge gained through study of the PCP system may also provide insights into the closely related fucoxanthin-system of diatoms, chrysophytes and brown algae.

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FINAL TECHNICAL REPORT

GRANT #: N00014-88-K-0060 R&T CODE: 4412028---03

PRINCIPAL INVESTIGATORS: Dr. Barbara Prezelin/ Dr. Edward Triplett
GRANT TITLE: Molecular Biology of the Photoregulation of Photosynthetic Light-Harvesting Complexes in Marine Dinoflagellates
PERIOD OF PERFORMANCE: 1 October 1987-31 March 1991

OBJECTIVE: Our long term objective is to study the genetic basis of light- and nutrient- regulation of photosynthetic light harvesting complexes in marine dinoflagellates.. The peridinin-chorophyll a-protein (PCP) complexes of Glenodinium sp.are being developed as a model system for the proposed genetic analyses. The specific aims of the present proposal are to obtain complete sequences of all cDNA molecules representing the translatable portions of the PCP gene family and of all PCP genes along with their upstream areas of control. PCP light controlled genes will then be studied with particular attention to the nature of the information transfer which leads from the perception of a light signal to an altered pattern of transcription of PCP genes.

ACCOMPLISHMENTS: We now have a firm foundation both at the physiological level and at the molecular biological level for studying the genetic basis of light regulation of light harvesting components (LHC) in a broad sprectrum of eucarylotic algae. We have used Heterocapsa pigmaea (AKA Glenodinium sp.) as a model system to 1) perform a DNA analysis including renaturing kinetics and melting profiles, 2) produce both genomic and cDNA libraries, 3) select from the cDNA library over 30 clones for the PCP mRNA, 4) select from the genomic library six clones carrying sequences for the PCP DNA, 5) perform southern blots with nuclear and Chloroplast DNA to determine that PCP is represented by a nuclear encoded multigene (at least six) family, 6) perform northern blots on mRNA extracted from cells grown under different light conditions and from this determine that there are two size classes of PCP mRNA, one of which is light regulated and one of which appears to be constitutive, 7) obtain the nucleotide sequences of one complete and five truncated PCP cDNA's, 8) determine that these six cDNA's are not identical (and therefore encoded by different genes) but share extensive homology with each other. they appear to be totaly unrelated to light harvesting genes of flowering plants, euglena and cyanobacteria (blue green algae).

SIGNIFICANCE: Our results are providing new approaches for studying the structure and function of light-harvesting complexes within the photosynthetic apparatus of these microalgae, as well as molecular explanations for the

nutrient dependent photoadaptive physiology of microalgae thus far only well defined at the whole cell level. In addition, our information provide the first key steps toward broadening the use of marine microalgae and their products in modern industry. Furthermore, field populations can often reach cell densities and develop natural toxin to the extent that they become a threat to water quality control. Understanding how light fields and and nutrient conditions control the genetic expression of important light harvesting components should lead to improved approaches to regulating algal growth yield in contained environments.

WORK PLAN: The planned experiments are: a) to sequence the complete set of PCP cDNA's that we have isolated from cDNA libraries. Sequence comparisons will give us the exact number of genes in the family. b) Oligonucleotides directed to unique sequences in our cloned cDNA's will be used to perform northern blots on RNA prepared from low and high light cultures. These experiments will tell us which genes respond to low light by producing higher levels of mRNA and which are constitutive. c) Nuclear runoff experiments will be performed on nuclei derived from low light and high light cultures. These experiments will tell whether the control of light regulated genes is at the transcriptional or posttranscriptional level. d) PCP genes found to be transcriptionally regulated by light are to be completely sequenced . e)Transcriptionally controlled PCP genes would be further analyzed for cis operating control regions by gel retardation assays and DNAse footprinting. f) A comparison will be made between PCP genes of H. pigmaea and those of other dinoflagellates having different life styles for optimizing the capture of radiant energy. g) We shall continue our studies on the effects of light levellight color and nutrient availability on the kinetics of transcription and translation of the PCP genes. h) We shall begin studies on genes involved in coding for the second component (chlorophyll a/c_2 proteins) of the H. pigmaea light harvesting system.

INVENTIONS: none

PUBLICATIONS AND REPORTS:

Roman S., N. Nadathur, E. Triplett, and B. Prezelin. 1988. Light regulation of peridinin-chlorophyll a-protein complexes in the dinoflagellate Peridiniun ap. use of anti-PCP antibodies to detect PCP gene products in cells grown in different light conditions. Plant Physiol. 88:594-599

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Roman S., E. Triplett and B. Prezelin (1988) Use of anti-PCP antibodies to quantify PCP apoproteins and to indicate changes in mRNA species in Glenodinium sp. grown under different light conditions. Presented at the Ocean Sciences Meeting (AGU/ASLO), New Orleans, LA.

Chang. S, S. Govind, E. Triplett and B. Prezelin (1988). Molecular biology of the dinoflagellate Glenodinium sp.: initial isolation and characterization of cDNA clones encoding the apoprotein of peridin- chlorophyll a-protein complexes. Presented at the Ocean Sciences Meeting (AGU/ASLO), New Orleans, LA.

Govind, N., S. Chang, S. Roman, E. Triplett and B. Prezelin (1988). Molecular biology of the marine dinoflagellate Glenodinium sp.: Use of anti-PCP antibodies to isolate and characterize cDNAs encoding the apoprotein of peridin-chlorophyll a-protein complexes. Presented at the "Chromophyte Algae" Meeting, Plymouth England.

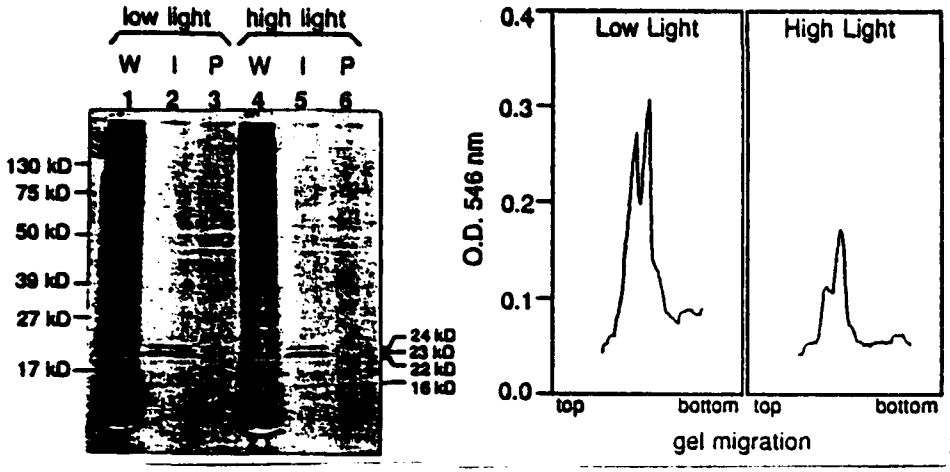
Roman S., N. Govind, E. Triplett, R.Trench, B. Sweeney and B. Prezelin (1988) Specificity of Immunological cross-reactivity of anti-PCP antibodies with light harvesting peridinin-chlorophyll protein complexes purified from free-living and symbiotic dinoflagellates. Presented at the San Francisco winter meeting of the American Society of Limnology and Oceanography (ASLO) EOS 69 (44):1099

Nelson N. and B. Prezelin (1988) Light induced variations in specific absorption, pigmentation and wavelength dependent package effect in the dinoflagellate Glenodinium sp. Amer. Soc. for Limnol. and Oceanog. San Fransisco, December 1988 EOS 69(44):1107-1108

Manuscripts Submitted

Nadathur, G., E. Triplett, S. Roman and B. Prezelin. (1990). Characterization of DNA from the Dinoflagellate Glenodinium sp.

Govind N., E. Triplett, S. Roman, S. Chang, and B. Prezelin (1990) cDNA clones encoding the peridinin-chlophyll-protein (PCP) complex aprotein from Glenodinium sp. : isolation and characterization



immunoprecipitation of PCP apoprotein from in-vitro translation reactions supplied with total RNA from high light and low light cultures

Objectives

Regulation of Dinoflagellate Light Harvesting Complexes

- Complete sequences of all cDNA's representing the light harvesting proteins
- Sequence PCP genomic DNA including upstream areas of transcriptional control
- Study nature of information transfer that leads from light signal to altered pattern of transcription of PCP genes

Accomplishments

- DNA analysis, including renaturation kinetics and melting profiles
  Produced genomic and cDNA libraries
- Selected 30 cDNA clones for PCP DNA
  Selected 6 genomic PCPclones
- Determined that one PCP gene is transcriptionally regulated by light
- Obtained nucleotide sequences of 6 PCP cDNA's
- determined that PCP is a multigene family

Significance

- Provide new approaches for studying the structure and function of light harvesting complexes in microalgae
- provide molecular expanations for nutrient dependent photoadaptive physiology of microalgae
- Broaden the use of marine algae and their product in modern industry